Folate intake and incidence of hypertension among American young adults: a 20-y follow-up study[1–3]

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ABSTRACT

Background: Laboratory studies suggest that folate intake may decrease blood pressure (BP) through increasing nitric oxide synthesis in endothelial cells and/or reducing plasma homocysteine concentrations. However, human studies, particularly longitudinal data, are limited.

Objective: Our objective was to investigate whether dietary folate intake is associated with the 20-y incidence of hypertension.

Design: We prospectively followed 4400 men and women (African Americans and whites aged 18–30 y) without hypertension at baseline (1985) in the Coronary Artery Risk Development in Young Adults study 6 times, in 1987, 1990, 1992, 1995, 2000, and 2005. Diet was assessed by dietary-history questionnaire at baseline and in 1992 and 2005. Incident hypertension was defined as the first occurrence at any follow-up examination of systolic BP $\geq$140 mm Hg, diastolic BP $\geq$90 mm Hg, or use of antihypertensive medication.

Results: A total of 989 incident cases were identified during the 20-y follow-up. After adjustment for potential confounders, participants in the highest quintile of total folate intake had a significantly lower incidence of hypertension (HR: 0.48; 95% CI: 0.38, 0.62; *P*-trend $<$ 0.01) than did those in the lowest quintile. The multivariable HRs for the same comparison were 0.33 (95% CI: 0.22, 0.51; *P*-trend $<$ 0.01) in whites and 0.54 (95% CI: 0.40, 0.75; *P*-trend $<$ 0.01) in African Americans (*P*-interaction = 0.047). The inverse associations were confirmed in a subset of the cohort (n = 1445) with serum folate measured at baseline and in 1992 and 2000.

Conclusions: Higher folate intake in young adulthood was longitudinally associated with a lower incidence of hypertension later in life. This inverse association was more pronounced in whites. Additional studies are warranted to establish the causal inference. *Am J Clin Nutr* 2012;95:1023–30.

INTRODUCTION

Hypertension, a health disorder and a major risk factor for other chronic diseases, has become an important public health challenge worldwide (1–3). Therefore, identifying potential protective or risk factors for hypertension is of great public health significance.

Laboratory studies suggest that folate intake may have beneficial effects on blood pressure (BP)[4] by increasing nitric oxide synthesis in endothelial cells and/or reducing plasma homocysteine concentrations (4, 5). A meta-analysis of 12 small (from 17 to 100 participants), randomized controlled trials showed that high-dose ($\geq$5000 μg/d) folic acid supplementation for 2 to 16 wk can significantly lower systolic BP with a mean reduction of −2.03 mm Hg (95% CI: −3.63, −0.43) (6). Another placebo-controlled study indicated that high-dose (15 mg/d) folate administration for 3 wk significantly decreased both systolic and diastolic BP in postmenopausal women (7). However, longitudinal data are limited. One 8-y follow-up study using data from the Nurses' Health Study I (subjects aged 27–44 y) and II (subjects aged 43–70 y) is the only published large-scale study in which an inverse association between folate intake and incident hypertension (defined based on self-reported BP) was documented (8). Therefore, we prospectively examined folate intake in relation to incidence of hypertension in a large biracial cohort of American men and women over 20 y follow-up using data from the Coronary Artery Risk Development in Young Adults (CARDIA) study.

SUBJECTS AND METHODS

Study population

The CARDIA study is an ongoing institutional review board–approved, biracial prospective cohort study designed to examine

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[4] Abbreviations used: BP, blood pressure; CARDIA, Coronary Artery Risk Development in Young Adults; CVD, cardiovascular disease; PA, physical activity.

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the evolution of cardiovascular disease (CVD) risk factors among 5115 young adults aged 18–30 y at baseline in 1985. Participants were enrolled from 4 US metropolitan cities: Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA. Sampling was designed to achieve approximately equal distributions across age (18–24 or 25–30 y), sex, race (African American or white), and education (high school or less, greater than high school). The details of the study design and recruitment protocol were described elsewhere (9). To date, 6 follow-ups have been conducted, in 1987, 1990, 1992, 1995, 2000, and 2005. Follow-up rates averaged >90%, and ~70% of the participants in the original cohort returned in 2005. The study was approved by the institutional review boards of the centers involved. All participants provided written informed consent.

In the current study, exclusions included missing data on folate intake (n = 4), implausible total energy intake (<800 or >8000 kcal/d for men and <600 or >6000 kcal/d for women; n = 128), and diagnosed hypertension (n = 140) at baseline. Participants were also excluded if they lacked information for defining hypertension (n = 177) or were pregnant at any examination (n = 226). After these exclusions, 4440 participants remained in the analysis.

Ascertainment of folate intake

The CARDIA dietary-history questionnaire, an interviewer-administered quantitative food-frequency questionnaire, was designed to assess habitual eating patterns. The validity and reproducibility of the CARDIA dietary history have been evaluated and discussed elsewhere (10, 11). Dietary assessment was conducted 3 times at baseline and examination year 7 and year 20. Participants were asked to recall their usual dietary intakes over the past month. They were asked general questions about their diet, which elicited specific foods consumed in an open-ended fashion. They were then asked to report the frequency, amount of food consumption, and method of preparation for each item named. Information on folate-containing supplements was also collected. Values for total folate and other B vitamin intake included dietary and supplemental sources. Folate intake was measured 3 times at baseline and follow-ups in 1992 (year 7) and 2005 (year 20), and intakes of vitamins B-6 and B-12 were assessed in 1992 and 2005.

Ascertainment of serum folate, vitamin B-6, vitamin B-12, and homocysteine concentrations

Serum samples were collected from baseline and in 1992 and 2000 in a subset of the cohort (n = 1445) and stored at −70°C until analysis. Serum folate and vitamin B-12 were measured on the Hitachi 911 (Roche Diagnostics) by using the CEDIA homogeneous enzyme immunoassay system (Boehringer Mannheim). Intra- and interassay CVs were 4.3% and 6.7% for folate and 4.1% and 6.2 for vitamin B-12. Serum vitamin B-6 was measured by using a radioenzymatic assay (American Laboratory Products). Intra- and interassay CVs were 6.3% and 10.1%, respectively. Serum homocysteine was measured by a fluorescence polarization immunoassay (IMx Homocysteine Assay; Axis Biochemicals ASA) by using the IMx analyzer (Abbott Diagnostics) (12). The intra- and interassay CVs were 1.9% and 4.1%, respectively. All ascertainments were conducted in the Advanced Research and Diagnostic Laboratory, University of Minnesota.

The characteristics of the subset were generally similar to those of the entire cohort (data not shown). To assess how well folate intake represents body stores of folate, we calculated the Spearman correlation (r_s = 0.49) between the average of folate intake (dietary source plus supplemental source) and the mean concentration of serum folate in this subcohort.

Ascertainment of other covariates

Demographic variables, including age, sex, race, and education level, were collected by using a self-administered questionnaire and verified during clinic examinations. Smoking status was determined on the basis of self-report, and participants were classified as never, former, or current smokers. Current smokers were further classified into 3 groups based on the pack-year (0–4, 5–10, or ≥11 pack-years). Alcohol consumption was measured by using a validated questionnaire and was categorized into 4 groups: 0 (never drink), 0.1–14.9, 15.0–29.9, or ≥30 g/d physical activity (PA) was assessed by using the CARDIA Physical Activity History Questionnaire, an interviewer-administered self-report of frequency of participation in 13 categories of recreational sports, exercise, leisure, and occupational activities over the previous 12 mo. PA score was calculated in exercise units on the basis of the frequency and duration of activity over the previous year. A score of 100 exercise units is roughly equivalent to engaging in vigorous activity for 2 to 3 h/wk, 6 mo of the year (13–15).

Ascertainment of hypertension

BP was measured at baseline and at the first 5 follow-up examinations by using the Hawksley random-zero sphygmomanometer (W. A. Baum Co) and at the seventh examination (year 20) by using the *OmROn* HEM907XL by trained and certified technicians (16). Three BP measurements were taken from the right arm of each participant at 1-min intervals after a 5-min seated rest. Systolic and diastolic BPs were recorded as phase I and V Korotkoff sounds through year 15. On the basis of a study of ~900 participants, we estimated systolic BP (random zero = 3.74 + 0.96 × observed *OmROn* systolic BP and estimated diastolic BP = 1.30 + 0.97 × observed *OmROn* diastolic BP at year 20 (17, 18). The second and third of these measurements were averaged for analysis.

Hypertension was defined by using the Seventh Report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7) cutoff as a systolic BP ≥140 mm Hg, a diastolic BP ≥90 mm Hg, or use of antihypertensive medication at that examination. Incident hypertension was defined at the first follow-up examination, at which a participant met ≥1 of the above 3 criteria.

Statistical analysis

All analyses were performed by using SAS 9.2 (SAS Institute Inc). Two-sided tests were used and $P \leq 0.05$ was considered statistically significant. Chi-square test, ANOVA, and Kruskal-Wallis test were used to compare the baseline characteristics of participants according to quintiles of folate intake within each race subgroup as appropriate.

TABLE 1
Baseline characteristics of the study population according to quintile of total folate intake stratified by race[1]

	African American men and women (n = 2226)				White men and women (n = 2214)				
	Q1 (n = 445)	Q3 (n = 446)	Q5 (n = 445)	P^2	Q1 (n = 442)	Q3 (n = 443)	Q5 (n = 443)	P^2	Total (n = 4440)
Total folate ($\mu g \cdot 1000\ kcal^{-1} \cdot d^{-1}$)	84.4 (73.6–91.8)[3]	142.7 (134.5–152.1)	296.6 (255.8–378.9)		109.1 (91.8–122.3)	200.8 (188.3–213.2)	394.8 (339.5–501.1)		169.5 (120.4–247.5)
SBP (mm Hg)	112.1 ± 10.1[4]	111.2 ± 9.5	108.9 ± 9.6	<0.01	111.2 ± 10.5	109.7 ± 10.2	106.8 ± 10.1	<0.01	109.9 ± 10.2
DBP (mm Hg)	68.4 ± 9.4	68.0 ± 9.1	67.3 ± 9.7	0.20	68.7 ± 9.7	68.5 ± 8.4	67.7 ± 8.3	0.35	68.2 ± 9.1
Age (y)	23.7 ± 4.0	24.2 ± 3.9	24.8 ± 3.6	<0.01	24.9 ± 3.5	25.5 ± 3.3	26.0 ± 3.3	<0.01	24.9 ± 3.6
Female (%)	42.0	55.6	74.4	<0.01	37.3	49.2	75.6	<0.01	5.31
Current smokers (%)	47.8	37.5	24.6	<0.01	45.6	27.4	16.7	<0.01	29.7
Education (y)	12.6 ± 1.7	13.0 ± 1.7	13.6 ± 1.9	<0.01	13.6 ± 2.4	14.7 ± 2.3	15.1 ± 2.2	<0.01	13.8 ± 2.3
Alcohol (mL/d)	4.8 (0.0–14.9)	2.4 (0.0–11.9)	0.0 (0.0–7.2)	<0.01	7.7 (0.0–24.3)	7.2 (0.0–19.3)	4.8 (0.0–13.6)	<0.01	4.8 (0.0–14.6)
Physical activity (EU)	311 (148–516)	303 (163–550)	321 (159–526)	0.10	366 (198–585)	395 (252–627)	410 (252–612)	0.01	362 (198–576)
BMI (kg/m^2)	25.4 ± 5.8	25.5 ± 6.1	25.2 (5.1)	0.62	24.4 (4.6)	23.3 (3.5)	23.1 (4.1)	<0.01	24.5 (5.0)
HOMA-IR	2.8 ± 1.8	2.8 ± 2.9	2.4 (1.4)	0.02	2.5 (1.6)	2.1 (1.1)	2.0 (1.5)	<0.01	2.4 (1.6)
Family history of hypertension (%)	58.7	54.5	53.0	0.28	45.9	44.7	44.7	0.24	49.5
Dietary intake									
Energy (kcal/d)	3422.0 ± 1611.5	2975.2 ± 1430.0	2517.7 ± 1208.8	<0.01	3107.7 ± 1373.6	2718.8 ± 1160.4	2117.5 ± 876.1	<0.01	2839.7 ± 1338.8
Sodium ($mg \cdot 1000\ kcal^{-1} \cdot d^{-1}$)	1430.9 ± 284.7	1443.3 ± 269.6	1415.3 ± 276.0	0.21	1535.9 ± 311.5	1539.4 ± 273.2	1530.9 ± 323.9	0.14	1492.8 ± 297.1
Linoleic acid (g/d)	22.4 ± 12.5	19.9 ± 11.3	17.5 ± 10.7	<0.01	20.8 ± 11.7	18.1 ± 9.0	14.8 ± 9.2	<0.01	19.2 ± 11.1
α-Linolenic acid (g/d)	2.3 ± 1.2	2.1 ± 1.2	1.8 ± 1.0	<0.01	2.2 ± 1.1	1.9 ± 1.0	1.5 ± 0.8	<0.01	2.0 ± 1.1
LCω3PUFAs (g/d)	0.10 ± 0.12	0.12 ± 0.15	0.14 ± 0.23	0.08	0.09 ± 0.12	0.11 ± 0.15	0.10 ± 0.12	0.02	0.11 ± 0.17

[1] DBP, diastolic blood pressure; EU, exercise unit; LCω3PUFAs, long-chain omega-3 PUFAs; Q, quintile; SBP, systolic blood pressure.
[2] Derived from a test of differences across all quintiles of folate intake, obtained by ANOVA, Kruskal-Wallis test, or chi-square test as appropriate.
[3] Median; IQR in parentheses (all such values, unadjusted).
[4] Mean ± SD (all such values, unadjusted).

Cox regression analysis was used to examine folate intake in relation to incident hypertension. Follow-up time was calculated as the difference between the baseline and the year when hypertension was first identified, examination year 20, or the year a participant was censored, whichever came first (19). To reduce measurement error caused by within-person variation and to best represent long-term dietary intake, we used cumulative average intake of nutrients or foods. For example, we related folate intake reported at baseline to new hypertension cases identified at year 2 and year 5 and the average of folate intake reported at baseline and year 7 and year 20 to new cases identified at year 20. We categorized exposures of interest into quintiles based on their distribution. We used a sequential covariate-adjusted strategy in the analysis: model 1 adjusted for age, sex, race, and study center, and model 2 additionally adjusted for BMI, education, smoking status, alcohol consumption, PA, family history of hypertension, and dietary intakes of total energy, sodium, potassium, calcium, α-linolenic acid, linoleic acid, and long-chain omega-3 (n−3) fatty acids. We also verified the association between cumulative average serum folate concentrations and incident hypertension in a subset of the cohort by using a similar sequential strategy.

In addition, we investigated whether race and sex modified the association, because some previous studies indicated that race and sex may modify the folate-CVD (20) or the homocysteine-hypertension relation (21). These analyses were performed by creating interaction terms for folate intake with these potential modifiers. The *P* values for interaction were calculated from a likelihood ratio test by comparing models with and without the interaction terms. Moreover, propensity scores were used to balance the observed covariates between participants from the study groups (eg, 2 extreme quintile groups) to mimic what happens in a randomized study in a sensitivity analysis (22). Furthermore, to explore potential mechanisms, we *1*) examined the relation between serum concentrations of folate and homocysteine by using Spearman partial correlation analysis, *2*) assessed homocysteine concentrations in relation to BP by using generalized estimating equations, and *3*) determined the association of homocysteine concentrations with incident hypertension by using the Cox regression model.

RESULTS

During 70,738 person-years of follow-up, 989 incident cases of hypertension were identified (incident rate = 0.014/person-year). Of them, 577 were newly diagnosed by research staff at follow-up visits, and 412 were those already diagnosed before a follow-up visit. At baseline, the mean (±SD) age of the study population was 24.9 ± 3.6 y, and the mean (±SD) BMI (in kg/m^2) was 24.5 ± 5.0. Considering that racial differences in the hypertension rate due to different lifestyles were previously determined in CARDIA (23), we described baseline characteristics of the study population stratified by race (**Table 1**). Compared with participants in the lowest quintile of folate intake, those in the highest quintile were slightly older, were more likely to be females and noncurrent smokers, and had a relatively higher education level and lower alcohol consumption among both African Americans and whites. In addition, those with a high folate intake were more likely to be active and lean among whites but not among African Americans.

As illustrated in **Table 2**, folate intake was inversely associated with incidence of hypertension. The inverse association became statistically significant from the second quintile and strengthened slightly from the third to fifth quintiles. Participants in the highest quintile of folate intake had a significantly lower incidence of hypertension (HR: 0.48; 95% CI: 0.38, 0.62; *P*-trend < 0.01) than did those in the lowest quintile after

TABLE 2
Multivariable-adjusted HRs (95% CIs) of incident hypertension by quintile of total folate intake[1]

	Quintile of energy-adjusted total folate intake					
	1 (lowest)	2	3	4	5 (highest)	*P*-trend[2]
Folate intake ($\mu g \cdot 1000\ kcal^{-1} \cdot d^{-1}$)[3]	91.8 (79.8–102.3)	129.6 (120.4–139.3)	169.5 (159.1–181.1)	226.4 (210.0–247.5)	353.0 (302.3–454.3)	
Overall cohort (*n* = 4440)						
No. of events/participants	276/888	240/888	180/888	142/888	151/888	
Model 1[4]	1.00	0.61 (0.51, 0.72)	0.43 (0.36, 0.53)	0.34 (0.28, 0.42)	0.38 (0.31, 0.47)	<0.01
Model 2[5]	1.00	0.63 (0.52, 0.76)	0.48 (0.38, 0.59)	0.41 (0.32, 0.52)	0.48 (0.38, 0.62)	<0.01
Whites (*n* = 2214)						
No. of events/participants	65/244	59/344	59/466	65/560	73/600	
Model 1[4]	1.00	0.38 (0.27, 0.55)	0.26 (0.18, 0.37)	0.22 (0.15, 0.31)	0.24 (0.17, 0.35)	<0.01
Model 2[5]	1.00	0.49 (0.33, 0.72)	0.30 (0.20, 0.45)	0.29 (0.19, 0.44)	0.33 (0.22, 0.51)	<0.01
African Americans (*n* = 2226)						
No. of events/participants	211/644	181/544	121/422	77/328	78/288	
Model 1[4]	1.00	0.67 (0.55, 0.82)	0.51 (0.40, 0.63)	0.39 (0.30, 0.51)	0.46 (0.35, 0.60)	<0.01
Model 2[5]	1.00	0.67 (0.54, 0.83)	0.54 (0.42, 0.69)	0.46 (0.34, 0.62)	0.54 (0.40, 0.75)	<0.01

[1] All models were constructed by using Cox proportional hazards regression analysis.
[2] Ordinal variables using medians in each quintile were created for the trend tests.
[3] Values are medians; IQRs in parentheses.
[4] Adjusted for age, sex, race (African American or white), and study center. Race was adjusted for only in the overall cohort.
[5] Additionally adjusted for BMI (continuous), physical activity (quintile), education (<12, 12–15.9, or ≥16 y), smoking status (nonsmokers, former smokers, or current smokers with pack-years between 1 and 4, between 5 and 10, or ≥11 y), alcohol consumption (0, 0.1–14.9, 15.0–29.9, or ≥30 mL/d), family history of hypertension (yes or no), and dietary intakes (quintiles) of total energy, sodium, potassium, calcium, α-linolenic acid, linoleic acid, and long-chain omega-3 fatty acids.

adjustment for potential confounders. The multivariable HRs for the same comparison were 0.33 (95% CI: 0.22, 0.51; P-trend $<$ 0.01) in whites and 0.54 (95% CI: 0.40, 0.75; P-trend $<$ 0.01) in African Americans (P-interaction = 0.047). Sex did not appreciably modify the association (P-interaction = 0.44).

We considered the potential effect of supplement use. When we further adjusted folic acid–containing supplement use status (yes or no), the inverse associations were essentially unchanged (data not shown). When dietary folate intake alone was used in the analysis, the results remained (data not shown). In addition, we stratified data by folic acid–containing supplement use status; our findings were generally consistent in both users and nonusers (**Table 3**).

As shown in **Table 4**, serum folate was inversely associated with incidence of hypertension in the subgroup with serum folate data available. The incidence of hypertension was reduced by 51% in participants in the highest quintile compared with those in the lowest quintile of serum folate concentrations (HR: 0.49; 95% CI: 0.33, 0.71; P-trend $<$ 0.01). When further adjusted for dietary intakes of total energy, sodium, potassium, calcium, α-linolenic acid, linolenic acid, and long-chain omega-3 fatty acids, the inverse association was essentially unchanged: whites (HR: 0.44; 95% CI: 0.25, 0.77; P-trend $<$ 0.01) and African Americans (HR: 0.48; 95% CI: 0.23, 1.00; P-trend $<$ 0.01; P-interaction = 0.27).

Several sensitivity analyses were conducted to test the robustness of our main findings. First, to balance the observed covariates, we used a propensity score method to compare the 2 extreme quintile groups as an example. The HR in the highest quintile of folate intake compared with the lowest quintile was 0.54 (95% CI: 0.40, 0.73; P-trend $<$ 0.01) with adjustment for propensity scores (quintiles), which were derived from the same covariates in the main analysis; the corresponding HR was 0.58 (95% CI: 0.40, 0.85; P-trend $<$ 0.01) when adjusted for propensity scores (quintiles) derived from the covariates in the final model with additional dietary variables (ie, magnesium, caffeine, protein, and fiber). Second, when we further adjusted baseline systolic blood pressure and glucose concentration in the model, the inverse association between folate intake and hypertension were essentially unchanged. Third, when we chose not to update dietary information for incident cases defined only on the basis of antihypertensive medication use at examination years 7 and 20, because these participants were more likely to change their dietary habits after receiving a diagnosis of hypertension, the results were not appreciably altered. Fourth, when we used the most-recent dietary information in the analysis, the results again did not change appreciable. Fifth, when we recalculated the person-time for the 412 cases that were diagnosed before a follow-up visit using the middle point between the current and last visits, the results were essentially unchanged. Finally, because vitamin B-6 and B-12 intakes were assessed only at examination years 7 and 20, we reexamined folate intake in relation to incidence of hypertension using examination year 7 as baseline. After adjustment for the same covariates in model 2 (Table 2), the HR (the highest compared with lowest quintile) was 0.48 (95% CI: 0.36, 0.65; P-trend $<$ 0.01). After further adjustment for intakes of vitamin B-6 and B-12, the inverse association remained.

To explore potential mechanisms, we examined the relation between serum folate and homocysteine concentrations and

TABLE 3
Multivariable-adjusted HRs (95% CIs) of incident hypertension by quintile of total folate intake stratified by folic acid–containing supplement use status[1]

	Quintile of energy-adjusted folate intake					
	1 (lowest)	2	3	4	5 (highest)	P-trend[2]
Folic acid–containing supplement users (n = 2255)						
Folate intake ($\mu g \cdot 1000\ kcal^{-1} \cdot d^{-1}$)[3]	141.2 (119.6–157.3)	192.2 (181.0–202.6)	237.7 (224.7–249.8)	295.7 (280.8–316.2)	446.2 (382.2–560.1)	
No. of events/participants	168/451	93/451	76/451	83/451	66/451	
Model 1[4]	1.00	0.52 (0.40, 0.67)	0.41 (0.31, 0.55)	0.47 (0.36, 0.61)	0.38 (0.28, 0.51)	<0.01
Model 2[5]	1.00	0.58 (0.44, 0.76)	0.51 (0.38, 0.69)	0.59 (0.44, 0.80)	0.53 (0.38, 0.75)	<0.01
Folic acid–containing supplement nonusers (n = 2185)						
Folate intake ($\mu g \cdot 1000\ kcal^{-1} \cdot d^{-1}$)[3]	81.2 (70.5–87.3)	104.7 (98.8–110.0)	126.1 (119.3–131.6)	150.9 (143.6–158.6)	196.3 (178.9–226.2)	
No. of events/participants	122/437	111/437	98/437	99/437	73/437	
Model 1[4]	1.00	0.65 (0.51, 0.85)	0.50 (0.38, 0.65)	0.45 (0.35, 0.59)	0.38 (0.28, 0.51)	<0.01
Model 2[5]	1.00	0.65 (0.50, 0.85)	0.48 (0.36, 0.65)	0.44 (0.32, 0.60)	0.36 (0.25, 0.53)	<0.01

[1] All models were constructed by using Cox proportional hazards regression analysis.
[2] Ordinal variables using medians in each quintile were created for the trend tests.
[3] Values are medians; IQRs in parentheses.
[4] Adjusted for age, sex, race (African American or white), and study center.
[5] Additionally adjusted for BMI (continuous), physical activity (quintile), education (<12, 12–15.9, or ≥16 y), smoking status (nonsmokers, former smokers, or current smokers with pack-years between 1 and 4, between 5 and 10, or ≥11 y), alcohol consumption (0, 0.1–14.9, 15.0–29.9, or ≥30 mL/d), family history of hypertension (yes or no), and dietary intakes (quintiles) of total energy, sodium, potassium, calcium, α-linolenic acid, linoleic acid, and long-chain omega-3 fatty acids.

TABLE 4
Multivariable-adjusted HRs (95% CIs) of incident hypertension by quintile of serum folate in a subset of the cohort (n = 1445)[1]

	Quintile of serum folate					
	1 (lowest)	2	3	4	5 (highest)	P-trend[2]
Serum folate (nmol/L)[3]	4.20 (3.20–4.83)	6.53 (5.95–6.97)	8.67 (8.03–9.20)	11.17 (10.40–12.10)	15.97 (14.33–18.80)	
No. of events/participants	129/290	79/288	52/287	58/291	45/289	
Model 1[4]	1.00	0.52 (0.39, 0.69)	0.37 (0.27, 0.52)	0.44 (0.32–0.60)	0.39 (0.27–0.56)	<0.01
Model 2[5]	1.00	0.54 (0.40, 0.71)	0.40 (0.29, 0.56)	0.47 (0.34, 0.65)	0.47 (0.32, 0.68)	<0.01
Model 3[6]	1.00	0.56 (0.42, 0.75)	0.42 (0.30, 0.59)	0.51 (0.37, 0.71)	0.49 (0.33, 0.71)	<0.01
Model 4[7]	1.00	0.56 (0.42, 0.75)	0.43 (0.31, 0.61)	0.52 (0.37, 0.73)	0.55 (0.37, 0.82)	<0.01

[1] All models were constructed by using Cox proportional hazards regression analysis. Because serum biomarker was used as exposure, we considered model 3 as the final model.
[2] Ordinal variables using medians in each quintile were created for the trend tests.
[3] Values are medians; IQRs in parentheses.
[4] Adjusted for age, sex, race (African American or white), and study center.
[5] Additionally adjusted for BMI (continuous), physical activity (quintile), education (<12, 12–15.9, or ≥16 y), smoking status (nonsmokers, former smokers, or current smokers with pack-years between 1 and 4, between 5 and 10, or ≥11 y), alcohol consumption (0, 0.1–14.9, 15.0–29.9, or ≥30 mL/d), and family history of hypertension (yes or no).
[6] Additionally adjusted for serum concentrations (quintiles) of vitamin B-6 and vitamin B-12.
[7] Additionally adjusted for dietary intakes (quintiles) of total energy, sodium, potassium, calcium, α-linolenic acid, linoleic acid, and long-chain omega-3 fatty acids.

found that serum folate concentrations were significantly inversely related to serum homocysteine concentrations. Spearman's partial correlation coefficient was −0.46 ($P < 0.01$) between serum folate and homocysteine with adjustment for age, sex, race, and study center. Also, serum homocysteine concentrations were positively associated with BP. Systolic and diastolic BP were higher by 0.63 mm Hg (95% CI: 0.13, 1.13; $P = 0.01$) and 0.55 mm Hg (95% CI: 0.14, 0.96; $P < 0.01$), respectively, with a 5-μmol/L increment in serum homocysteine (~1 SD) with adjustment for potential confounders. In addition, higher homocysteine concentrations were also associated with elevated incidence of hypertension (data not shown).

DISCUSSION

In this 20-y follow-up prospective study, we found an inverse association between folate intake and incidence of hypertension, and this inverse association was stronger in whites than in African Americans. This inverse association was fairly robust and further supported by serum folate data from a subset of participants. Our findings are less likely to be explained by residual confounding or confounding by unmeasured factors, because the results persisted with adjustment for many potential confounders and in various sensitivity analyses.

Our findings agree with those from previous studies. An inverse relation between folate intake and incidence of hypertension was found in the Nurses' Health Study, although it was exclusively conducted in women (8). In addition, some clinical studies have shown that high-dose supplemental folic acid may be associated with reduced BP (7, 24, 25). For example, a small scale study documented an average drop in systolic and diastolic BP of 4.5 and 5.3 mm Hg, respectively, in postmenopausal women who received a daily dose of 15 mg of 5-methyltetrahydrofolate for 3 wk (7). Yet, there is also some contradictory evidence. A meta-analysis of 8 randomized trials concluded that dietary supplementation with folic acid had no significant effects on cardiovascular events within 5 y (26). However, the outcome in this meta-analysis referred to major coronary events and stroke and not specifically to hypertension. Also, short-term follow-up clinical trials only focusing on supplement use cannot rule out a long-term effect (ie, 20 y) of folate intake from both foods and supplements, as found in this study.

It was hypothesized that the potential antihypertensive effect of folate intake may be mainly due to a reduction in blood homocysteine concentrations through remethylation of homocysteine back to methionine (27). However, the findings from our study seemed not to support this hypothesis. When we further controlled for serum homocysteine in model 3 (Table 4), the association (quartile 5 compared with quartile 1) was slightly attenuated. Previous studies also suggested other mechanisms, such as an interaction with endothelial nitric oxide synthase and endothelium-derived hyperpolarizing factor, which may improve endothelial function independent of its homocysteine-lowering effect (28). Nonetheless, we do not have data from the current study to test these hypotheses. Further studies are needed to elucidate the mechanisms.

In this study, we found that race significantly modified the inverse association between folate intake and hypertension, with a greater benefit in whites. Race was also indicated to modify the folate-CVD relation in a previous study (20). In our study, this modification effect may be explained, at least partially, by the higher intake of folate in whites [median (25th–75th percentiles): 200.9 (144.9–282.2) $\mu g \cdot 1000\ kcal^{-1} \cdot d^{-1}$] than in African Americans ([142.7 (106.9–204.5) $\mu g \cdot 1000\ kcal^{-1} \cdot d^{-1}$] given the dose-response relation between folate intake and hypertension.

One concern is the accuracy of dietary assessment (29). The food-frequency questionnaire used in the current study, however, has been validated and shown to reasonably reflect long-term dietary intake (30). Also, intakes of many nutrients have successfully predicted CVD risk factors, including hypertension in previous CARDIA studies (17, 18, 31–33). In addition, possible measurement error in dietary assessment was somewhat reduced by using repeated measurements and cumulative average diet intakes. Moreover, the Spearman correlation coefficients of folate intake with serum folate concentrations at different visits ranged

from 0.37 to 0.59, which are reasonably high and further support the validity of our dietary assessment.

Another concern is that the observed inverse associations may reflect consumption of other potentially beneficial components of foods rich in folate, such as green leafy vegetables, or plant food patterns, including fruit, vegetables, nuts, legumes, and whole- and refined-grain products (34). However, the serum folate data confirmed the potential beneficial effect of folate on BP.

Other limitations also need to be considered. First, data on vitamins B-6 and B-12 intakes were unavailable in 1985, which limited our ability to consider the intakes of these 2 vitamins as potential confounders in the main analysis. However, when we used exam year 7 as the baseline, similar inverse associations between cumulative average folate intake and incidence of hypertension were documented after adjustment for these 2 vitamins in a subset of the cohort. Second, the generalizability of our findings may be limited because CARDIA was not a nationally representative sample.

A couple of other strengths enhanced the validity of this study. First, this study had a prospective design, a relatively large biracial sample, a 20-y follow-up, and especially that the participants at baseline were aged 18–30 y, which together enabled us to study the evolution of CVD risk by following the course of BP and the appearance of incident hypertension from young adulthood. Second, the BP of our participants was directly measured by trained personnel using standardized procedures, rather than being self-reported, which substantially reduced measurement error.

In conclusion, our findings provide prospective evidence that a higher intake of folate is associated with a lower incidence of hypertension. This inverse association was more pronounced in whites than in African Americans. Further studies are warranted to establish causal inference.

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